

TOP-DOWN PROTEIN SEQUENCING; THE USE OF TRAVELLING WAVE ION MOBILITY COUPLED WITH TOF MS FOR SEPARATING AND SEQUENCING MULTIPLY CHARGED PROTEIN IONS

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Introduction:

Electrospray mass spectrometry is a firmly established tool for the identification of proteins, via the analysis of complex tryptic peptide mixtures. It has also proven to be an extremely powerful technique for determining protein structure by mass analysis at the intact protein level. Often the complexity of the associated mass spectrum limits the information content that can be obtained from the data and additional stages of separation prior to analysis by mass spectrometry are desirable. The potential of using ion mobility spectrometry adds another, orthogonal, dimension of separation to the MS experiment, providing separation of species by their associated mobility, or drift time, a factor which is dependant upon ion size, shape and charge. Consequently, it is possible to separate co-eluting isobaric species which exhibit different drift times. We have combined a travelling wave ion mobility (TWIMS) device within a quadrupole orthogonal acceleration time-of-flight mass spectrometer, enabling ion mobility separations to be combined with electrospray mass spectrometry at high sensitivity.

We have investigated the potential of this configuration for the separation and subsequent mass analysis of multiply charged protein ions and to separate fragments derived from these multiply charged species, following CID. If mobility separation of fragment ions was efficient, this would reduce mass spectral complexity and facilitate detection and subsequent identification. In this study, ion mobility spectrometry (IMS) techniques have been used to separate the protein ion fragments. This technique will be compared to that of using a TOF based MS/MS approach alone, and the potential of IMS for biological applications will be discussed.